PATENT SPECIFICATION

NO DRAWINGS

1,029,222

Inventors: MARTIN COLE and MAURICE OSBURN MOSS

Date of filing Complete Specification: Nov. 6, 1964.

Application Date: Nov. 28, 1963. *No. 46965/63.*

Complete Specification Published: May 11, 1966.

Index at acceptance:—C2 A(1C2C, 2C1)

Int. Cl.:—C 07 d

COMPLETE SPECIFICATION

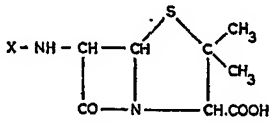
Derivatives of 6-Aminopenicillanic Acid

We, BEECHAM GROUP LIMITED, a British Company, of Beecham House, Great West Road, Brentford, Middlesex, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to new derivatives of 6-aminopenicillanic acid and is particularly concerned with compounds formed by the reaction of 6-aminopenicillanic acid and sugars.

We have now found that when 6-aminopenicillanic acid is added to solutions rich in certain sugars part of the β-lactam nucleus becomes stable to the action of penicillinase and that this is due to the formation of penicillinase-stable compounds.

Accordingly, the present invention provides derivatives of 6-aminopenicillanic acid of the general formula:—



(I)

where X is a monosaccharide or disaccharide glycosyl radical which may be substituted, said radical being derived from a sugar having a free aldehyde group.

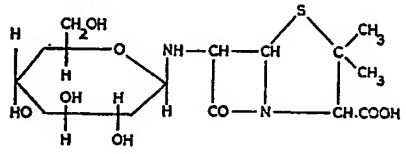
The compounds in which X in formula (I) is a glucose, maltose or lactose residue have been isolated as amorphous white powders from reaction mixtures containing only the sugar and 6-aminopenicillanic acid. The following Table sets out some of the properties of these compounds from which it can be seen that analysis of the components give a stoichiometry of one molecule of 6-aminopenicillanic acid combined with one molecule of sugar.

	6-Aminopenicillanic acid derivative		
	Glucose	Maltose	Lactose
% 6—A.P.A.	52%	30%	27%
% Sugar	40%	53%	49%
Molar ratio, Sugar: 6—APA	1.2 : 1	1.1 : 1	1.14 : 1
% Purity	96%	79%	70%
Decomp. Temp.	144—146°	128—140°	142—150°
Mobility toward anode	9 cm.	—	6 cm.
R_f BuOH/Pyridine	0.4	0.33	0.30

[*Price 4s. 6d.*]

After conversion to the free acid with Amberlite IR/120 (H+) resin ("Amberlite" is a Registered Trade Mark), 13.9 mg. of the glucose derivative took up 7.5 ml. of 3.9×10^{-3}N sodium hydroxide, giving an equivalent weight of 477. This result and the behaviour of these compounds on electrophoresis indicate that they are monobasic acids.

That the amino group of the 6-aminopenicillanic acid is involved in the formation of these compounds is further substantiated by the observation that there is no interaction between benzylpenicillin and reducing sugars. Neither is there any reaction between 6-aminopenicillanic acid and any sugar derivatives in which the aldehyde group is protected, such as sucrose, α-methyl-glucopyranoside and glucose 1-phosphate. It was confirmed by chromatography that acid hydrolysis liberates the free sugar unchanged and 6-aminopenicillanic acid only. The chemical properties outlined above and the mode of formation all confirm that these new compounds are N-glycosyl derivatives of 6-aminopenicillanic acid, for example in the case of the glucose derivative, β-N-glucosyl-6-aminopenicillanic acid of the general formula:—



(II)

The compounds of the present invention are all extremely soluble in water and insoluble in ordinary organic solvents.

The glycosyl, maltosyl and lactosyl compounds are all highly stable to staphylococcal penicillinase and decomposition in the presence of B. cereus penicillinase can only be demonstrated with difficulty. Thus, after 4 hours incubation at 37° and pH 7.0, a solution of 1 mg./ml. of the glucose derivative lost 28.5% of its β-lactam activity in the presence of penicillinase compared with a loss of 15% in its absence. Under the same conditions 6-aminopenicillanic acid itself is completely destroyed in 15 minutes.

Examples of substituted glycosyl radicals include those derived from glucose-6-phosphate and glucouronic acid.

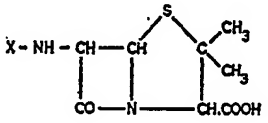
The following examples illustrates the present invention:—

EXAMPLE

Reaction mixtures containing 0.5% 6-aminopenicillanic acid and 4% glucose, maltose and lactose respectively were incubated at pH 6.0 and 26° C. for 48 hours. The residual 6-aminopenicillanic acid was removed on a column of Amberlite IR/120 (H+), the percolate being readjusted immediately to pH 6.5. The residual sugar was then removed by absorbing the 6-aminopenicillanic acid derivatives onto a column of De-Acidite FF resin ("De-Acidite" is a Registered Trade Mark) in its acetate form. The column was washed thoroughly with de-ionised water until no sugar could be detected in the washings. Material was eluted from the column with 0.5 M sodium salicylate, the eluate being collected in a number of fractions until the salicylate could be detected with ferric chloride. The fractions were assayed and those containing the bulk of penicillinase-stable β-lactam material were combined and stirred slowly into a 20-fold excess of cooled acetone. The white emulsion was centrifuged down and the resulting colourless gum treated with alcohol until it solidified into a white amorphous powder. After filtration the material was washed with acetone and dried thoroughly in an evacuated desiccator.

WHAT WE CLAIM IS:—

1. Derivatives of 6-aminopenicillanic acid of the general formula:—



wherein X is a monosaccharide or disaccharide glycosyl radical which may be substituted, said radical being derived from a sugar having a free aldehyde group.

2. Derivatives of 6-aminopenicillanic acid as claimed in claim 1, wherein X is a glucose, maltose or lactose residue.

3. A process for preparing derivatives of 6-aminopenicillanic acid according to claim 1, substantially as described with reference to the specific Example.

RONALD SMITHER,
Agent for the Applicants,
Chartered Patent Agent.

Leamington Spa: Printed for Her Majesty's Stationery Office, by the Courier Press (Leamington) Ltd.—1966. Published by The Patent Office, 25 Southampton Buildings, London, W.C.2, from which copies may be obtained.